



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Group Art Unit: Thai-An N. Ton

Kangsheng Wang

Examiner: 1632

Serial No. 09/781,046

Filed: February 8, 2001

For: A Method and System for  
Introducing a Gene into a Human Stem  
Cell

AFFIDAVIT OF KIRON KANGSHENG WANG

PURSUANT TO 37 C.F.R. § 1.132

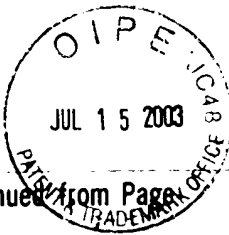
**APPENDIX A**

## **Linker Based Sperm-Mediated Gene Transfer Technology**

1. Over-Immunization of Balb/C Mice with Mouse Sperm Cells
2. Screen Hybridomas Which Does Not Prevent Sperm Fertilization by In Vitro Fertilization
3. Flow Cytometry Analysis of mAbs Bound to Mouse Sperm Cells
4. Generation of Transgenic Mice from Two Different Linkers mAb C and mAb D

## Over-Immunization of Balb/C Mice with Mouse Sperm Cells

TITLE



PROJECT NO.

BOOK NO.

1

Work continued from Page

1. Immunize 3 Balb/c mice with  $2 \times 10^6$  ~~B6D<sub>2</sub>F<sub>1</sub>~~ <sup>B6D<sub>2</sub>F<sub>1</sub></sup> and FVB male sperm mix with Ajuvant  
overimmune 8 time (twice/month) ( 2 for FVB sperm)  
1 for B6D<sub>2</sub>F<sub>1</sub> sperm)

- a. dissect epididymis of 12-15 weeks olds male, squeeze the sperm out from and let sperm in Modified Tyrode's medium without BSA.

- b. wash sperm with MTM three time <sup>count Number</sup> and immunize mix with 200ul TDM.

- c. Immunize <sup>5 weeks old</sup> Balb/c female. (twice/month)

Work continued to Page

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*Kangsheng Wang*

DATE 3/15/99

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DATE 10-1-99  
B 100 P ©

Screen Hybridomas Which Does Not Prevent Fertilization  
by In Vitro Fertilization

(screening assay shows that a number of hybridomas  
supernatants does not inhibit sperm cells bound with  
antibodies in the supernatant to fertilize oocytes.)

Work continued from Page

Test in vitro fertilization efficiency and blocking by

mAb

(hybridoma supernatant)

1. Set 20 egg/assay for IVF study, and

2. inject ~~with~~ 5 I.U. PMS (Sigma) ~~to~~ in 8 p.m. (day 1)50 B6D<sub>2</sub>F<sub>1</sub> female with  
8 weeks old3. 48 hours later, each mouse ~~was~~ was injected with 5 I.U. hCG (day 3)4. On day 4 at 7:30 a.m., ~~the~~ sacrifice the female mice and collect egg from with cumulus cell from swollen ampulla in MTM medium,5. ~~Add~~ Distribute each one clump of cumulus cell with egg to <sup>(average 20 cells)</sup> each ~~in~~ of 48 well dish, in 200  $\mu$ l MTM medium6. Add 20  $\mu$ l of supernatant of hybridoma to each well and incubate with  $5 \times 10^4$  sperm in 30  $\mu$ l MTM medium for 30 min7. add sperm mix to (5) <sub>in (6)</sub> and incubate in 37°C for 4 hr for in vitro fertilization8. ~~collect~~ collect and transfer <sup>fertilized</sup> eggs to CZB medium and incubate at 37°C for 20-22 hrs in ~~96 well~~9. observe the fertilization efficiency (++) <sup>No</sup> block fertilization ~~very~~ of sup. of hybridoma  
(++) block ~~at~~ some  
(+) block ~~was~~ a little  
blank blocked  
Work continued to Page

SCIENTIFIC BINDERY PRODUCTIONS CHICAGO 60605 MADE IN USA

SIGNATURE

Ken Wang

DATE

7/10/99

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DATE

10-1-99

Supernatant from  
hybridoma from mouse  
immunized with  
FVB and B6D2F1 mouse  
sperm

group 1 (+++ or +++)	group 2 (++)	group 3 (+)
1 B3	1 B8	1 A4
1 F5	1 C4	1 B5
2 D4	1 G10	1 F6
2 E8	2 B1	2 A10
3 C7	2 C8	2 B2
4 E7	2 E2	2 E3 (11)
	2 E4	2 F4
	3 A7	3 B7
	3 A10	3 D4
	3 B8	4 C8
	3 G2	4 C9

1	2	3	4	5	6	7	8	9	10	11	12
A1	A2	A4	A8	A10	B3	B4	B5	B6	B8	B9	C2
C4	C7	C8	C9	D4	D6	D8	E4	E5	E6	E12	F4
F6	F12	G2	G5	G8	G9	G10	H1	H5	H10	(H12)	A7
A10	A11	B1	B2	B3	B6	B7	B11	C3	C4	C5	C6
B8	C10	C11	D4	E2	E3	E4	E6	E7	E8	E9	F4
F5	F9	F10	F11	F12	G12	H7	H8	H10	(H12)	A1	A2
A7	A10	B1	B3	B7	B8	C3	C5	C7	C10	C11	D3
D4	D1	D10	E5	E1	E10	E3	F5	F6	F1	G2	G3

1	2	3	4	5	6	7	8	9	10	11	12
A4	A6	A12	H6	H9	(H11)	A2	A8	B2	B4	B6	B7
B12	C1	C7	C8	C9	D1	D5	D8	D10	D12	E2	E3
E5	E1	E7	E8	E9	E10	F1	F2	F3	F4	F6	F9
F10	F11	G3	G5	G6	G7	G10	NC				

# group 1 (### or ###) Retest

Free-time  
 ① show (### or ###) show ~~###~~ once  
 two-time twice

## group 2 (##)

1 A 7  
 1 C 5  
 1 C 12  
 1 F 3  
 1 H 12  
 2 B 10  
 2 D 5  
 2 D 8  
 2 E 10  
 3 A 6  
 4 C 10

## group 3 (+)

1 C 1  
 1 C 6  
 1 D 9  
 2 A 11  
 2 B 4  
 2 B 7  
 2 B 7  
 3 C 4  
 4 E 12  
 4 G 4

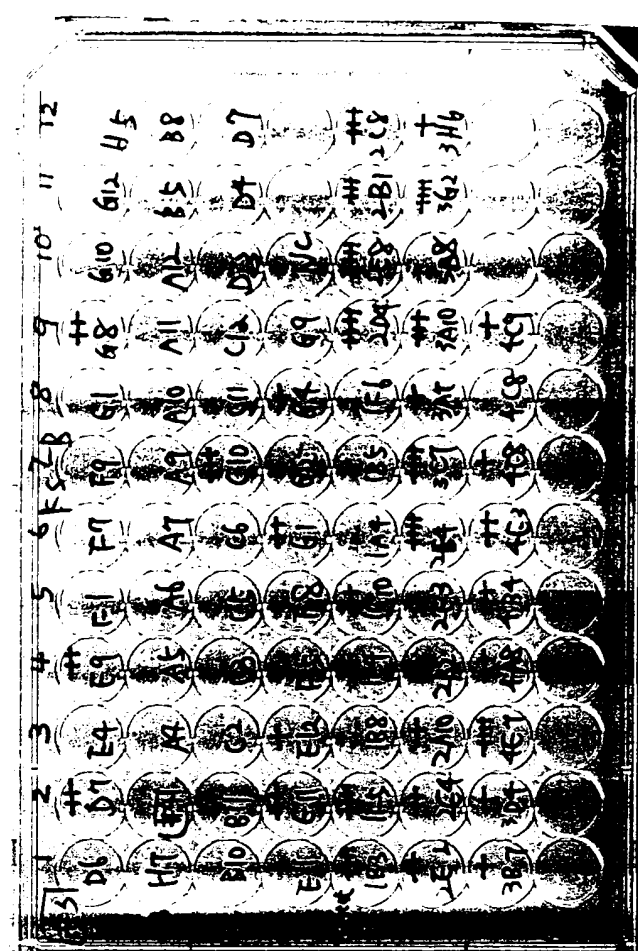
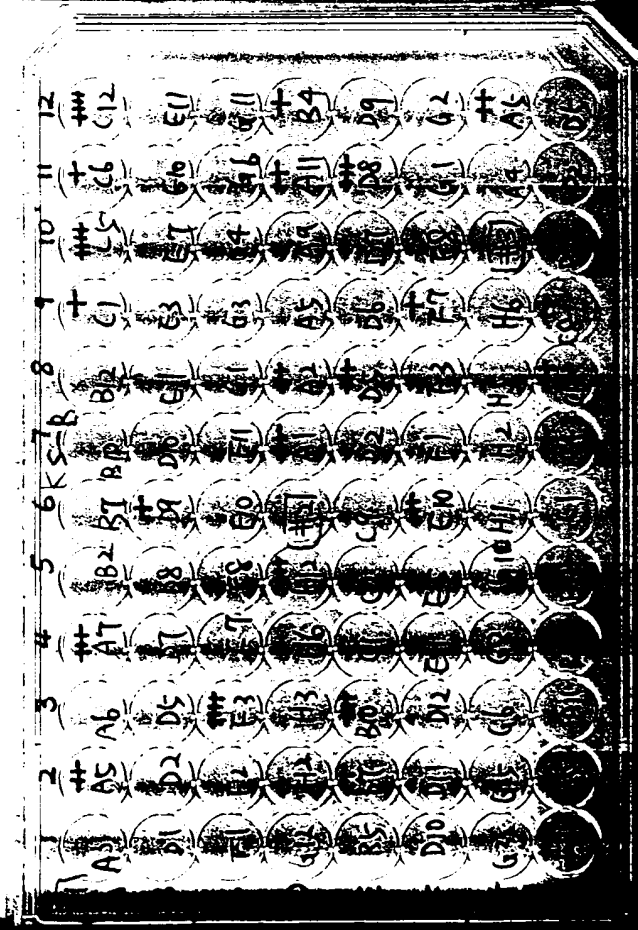
## Retest

② show ~~###~~ once  
 two-time twice

1 B 3  
 1 F 5  
 2 D 4  
 2 E 8  
 3 C 7  
 4 E 7

③ show ## twice

1 G 10  
 2 E 2  
 2 E 4  
 4 A 8  
 4 B 4  
 4 E 3





B subclone

1 B 3

Δ 1 A 8 (++) twice

2 A 2 (++) twice

2 E 7 (++) twice

3 C 7

Δ 2 C 5 (++) twice

1 D 8 (++) once (+) once

2 C 6 (++) once (+) once

2 D 10 (++) once (+) once

4 E 7

Δ 1 F 11 (++) twice

1 B 1 (++) twice

1 H 2 (++) twice

2 E 8 (++) twice

2 D 4

Δ 1 F 3 (++) once (+) once

1 G 4 (++) once (+) once

✓ 1 G 2 (++) once

✓ 1 G 9 (++) once

✓ 2 B 8 (++) once

✓ 2 C 3 (++) once

2 E 8

Δ 2 G 5 (++) twice

2 G 8 (++) twice

1 C 10 (++) twice

1 F 5

Δ 1 D 8 (++) once (+) once

✓ 1 H 4 (+) once

A 2 group

Δ 7 G 9 (++) once (+) once

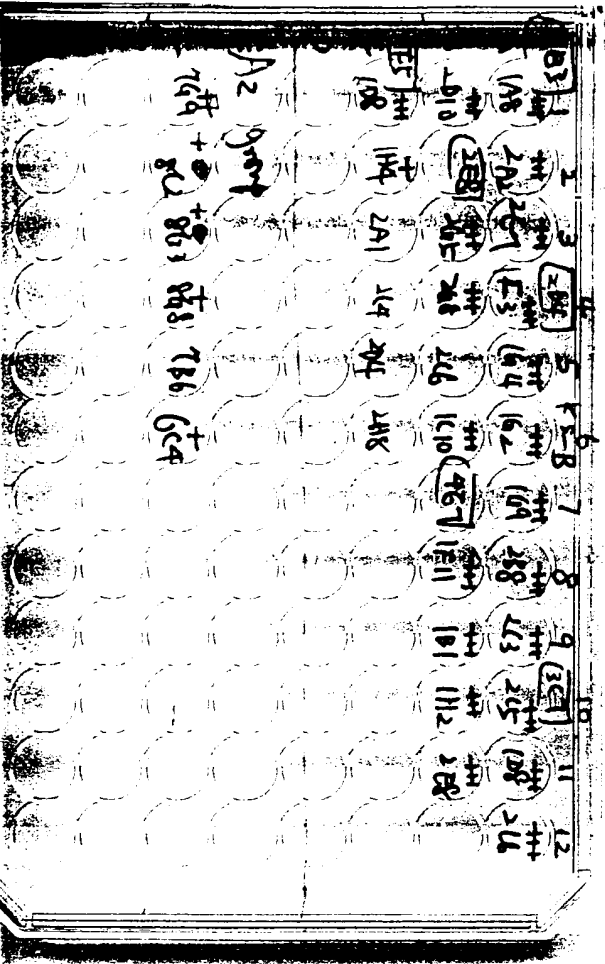
Δ 8 C 2 (+) twice

Δ 8 G 3 (+) twice

Δ 8 G 8 (++) once (+) once (0) once

Δ 6 C 4 (++) once (+) ~~once~~ twice

7 B 6 (+) twice (0) once



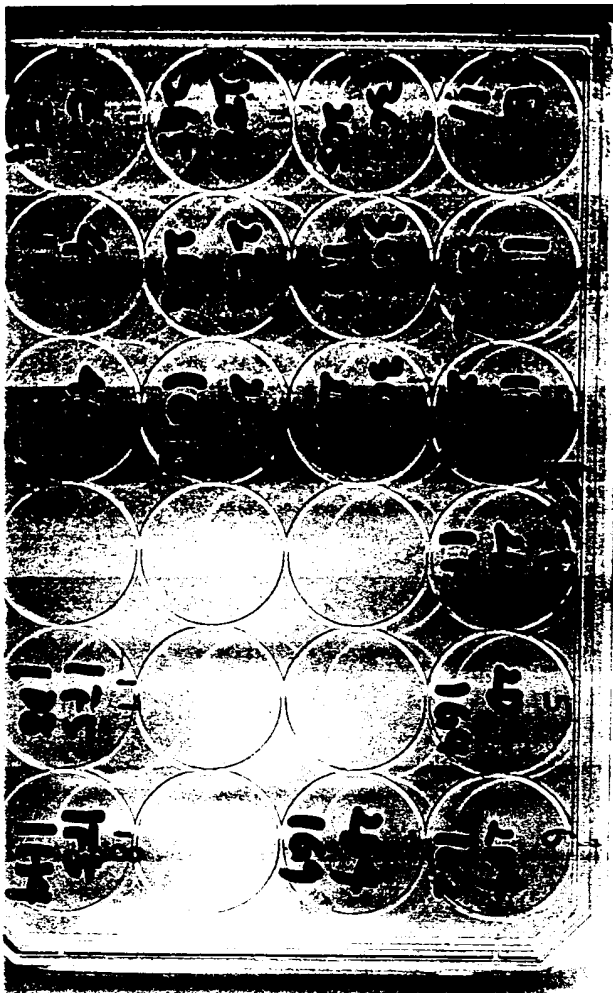
TITLE

PROJECT NO.

37

BOOK NO.

Work



B group do isotype assay, we got <sup>all</sup> IgM  
so we select

1B3	1A8 (A)
2D4	1F3 (B)
3C7	2C5 (C)
2E8	2G5 (D)
4E7	1F11 (E)
1F5	1D8 (F)

Work continued to Page

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DATE

8/5/99

DATE

10-1-99

## Flow Cytometry Analysis of mAbs Bound to Mouse Sperm Cells

(Four mAbs A, B, C and D show the binding of mouse sperm cells)

9/2

COW

BAG (Ken)  
FITC

2 Sep 99  
1530-1645

Hdk  
488(I22)/500nm

					N.
cattle	L1	P1	Log	P91	<del>580V</del>
90	L1	P2	Log	580V	PM T 1
ITC	L2	P3	Log	620V	3
Auto	L2	M	Log	550V	5

No Threshold

60.5/60.0

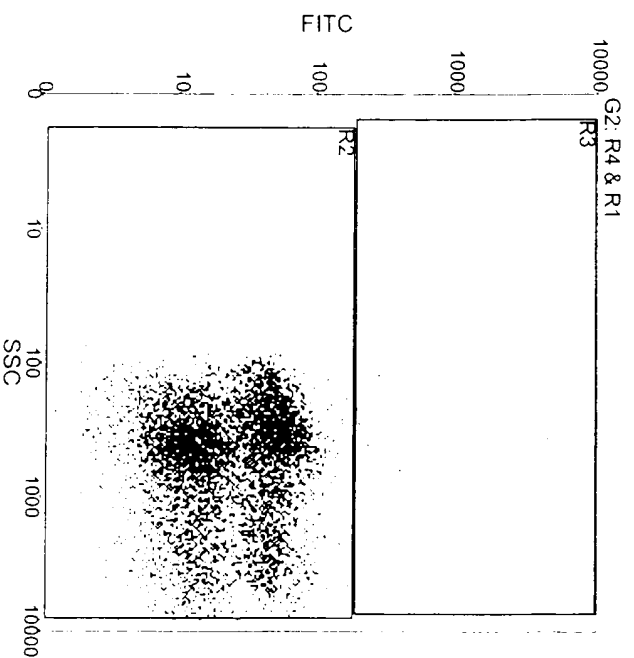
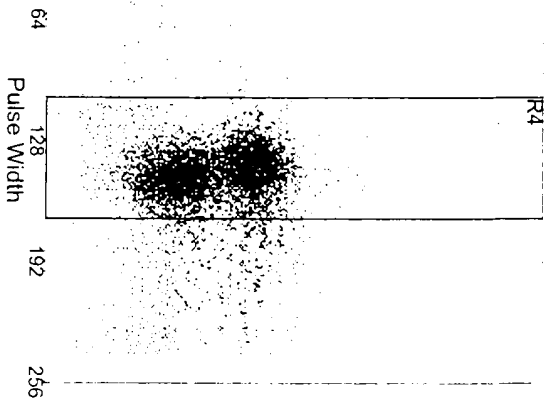
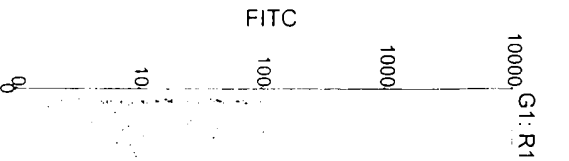
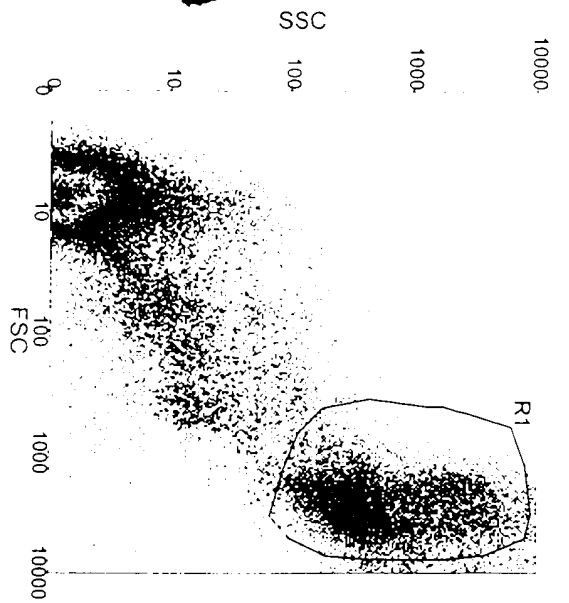
BAG-1999-09-02-000

Bovine Extender Auto

Bovine Sperm #4

-000	Bovine Extender	2nd only
-002	"	Myelin
-003	"	C
-004	"	D
-005	"	PBS Auto
-006	"	2nd only
-007	"	Myelin
-008	"	C
-009	"	D

-010	Mouse	Auto
-011	"	2nd only
-012	"	Myelin Tablin
-013	"	Myelin
-014	"	A
-015	"	B
-016	"	C
-017	"	D

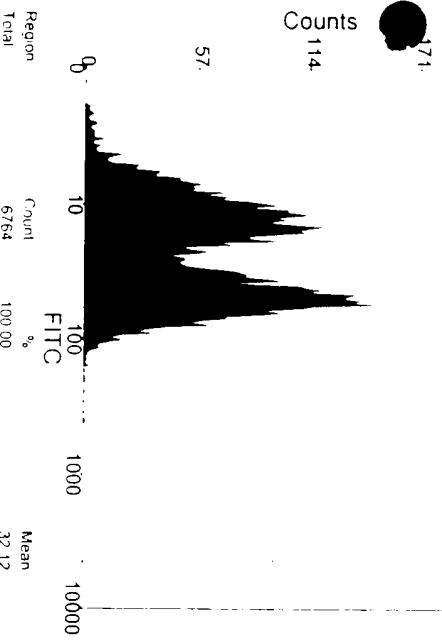


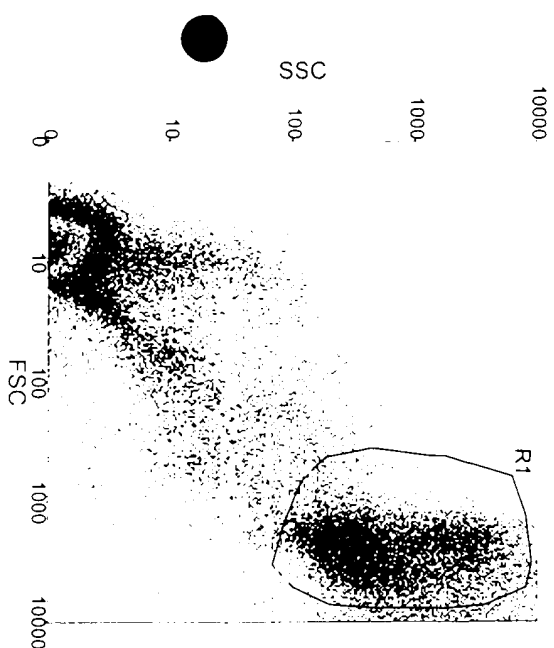
229 G2: R4 & R1

Region	Count	%	Mean
Total	50000	100.00	598.87, 192.30
R1	8385	16.77	2853.56, 898.98

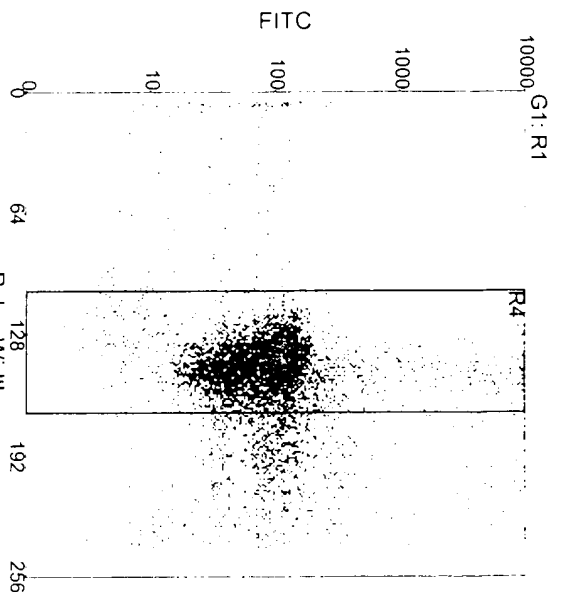
Region	Count	%	Mean
Total	8385	100.00	155.64, 32.50
R4	6764	80.67	143.52, 32.12

Region	Count	%	Mean
Total	6764	100.00	886.10, 32.12
R2	6753	99.84	886.72, 31.10
R3	11	0.16	507.41, 659.13

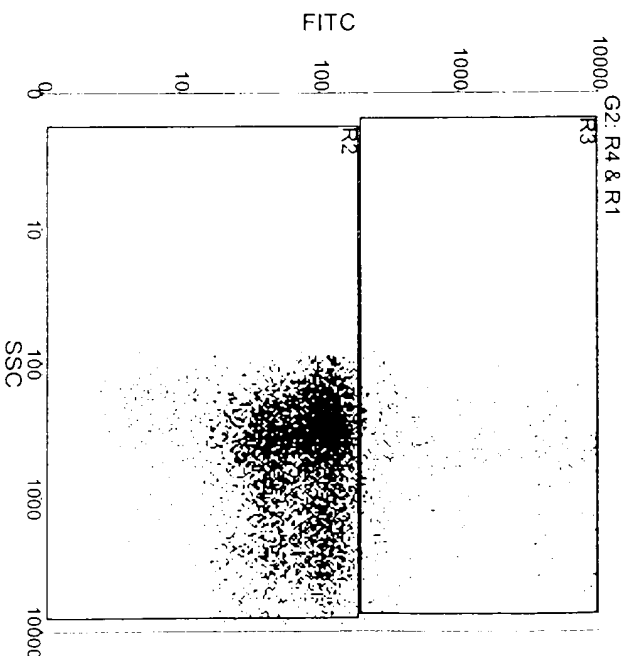




Region	Count	%	Mean
Total	50000	100.00	619.91, 204.17
R1	8714	17.43	2700.21, 895.86

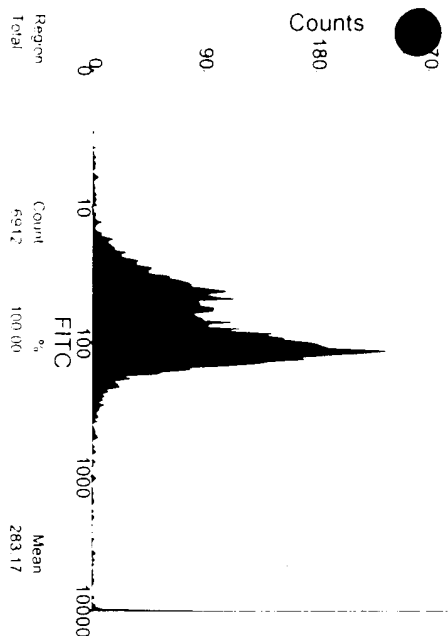


Region	Count	%	Mean
Total	8744	100.00	156.37, 300.47
R4	6912	79.05	142.58, 283.17

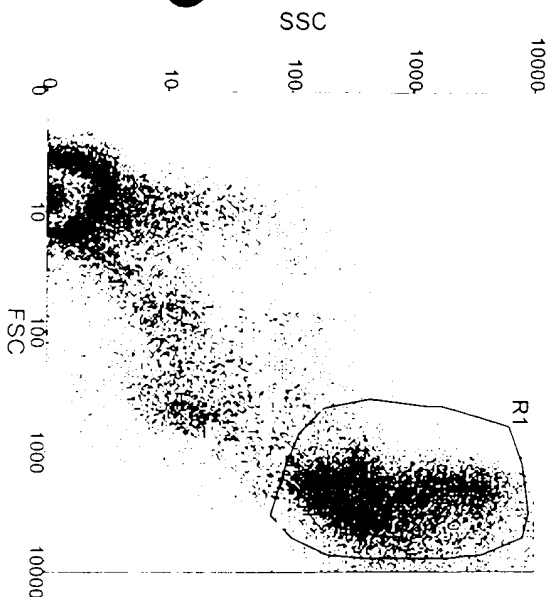


Region	Count	%	Mean
Total	6912	100.00	875.93, 283.17
R2	6464	93.52	863.97, 83.17
R3	349	5.05	1063.07, 1468.16

361 G2: R4 & R1

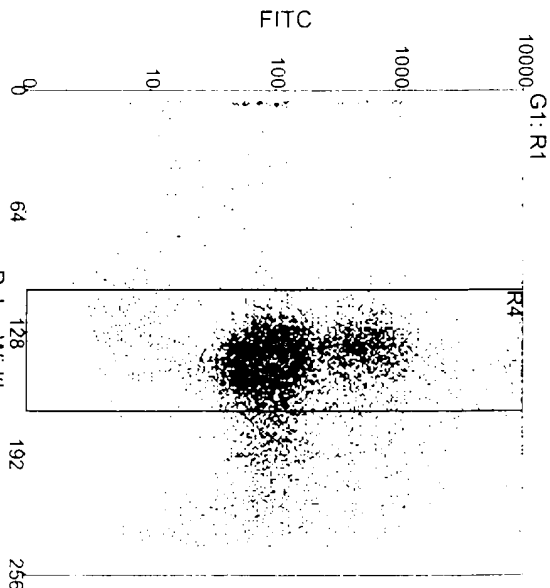


Region	Count	%	Mean
Total	6912	100.00	283.17

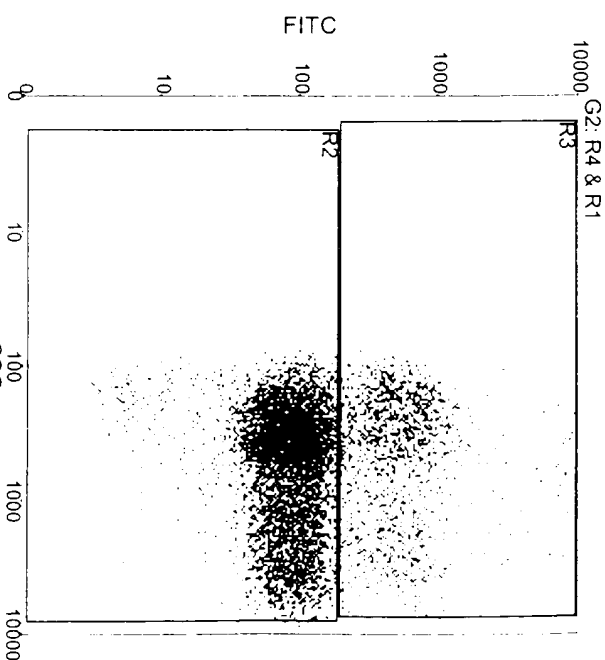


Region	Count	%	Mean
Total	50000	100.00	653.81, 221.67
R1	10891	21.78	2486.54, 886.74

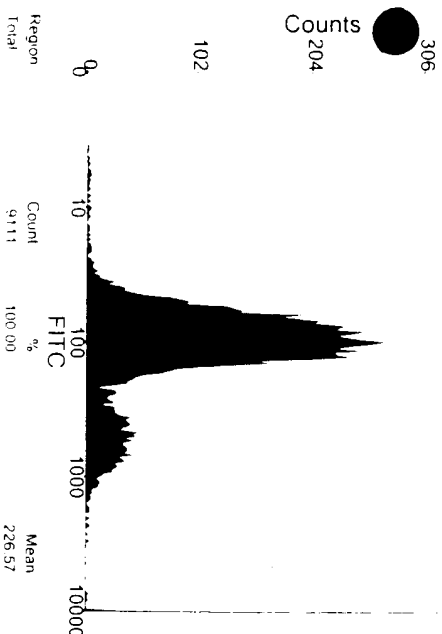
409 G2: R4 & R1



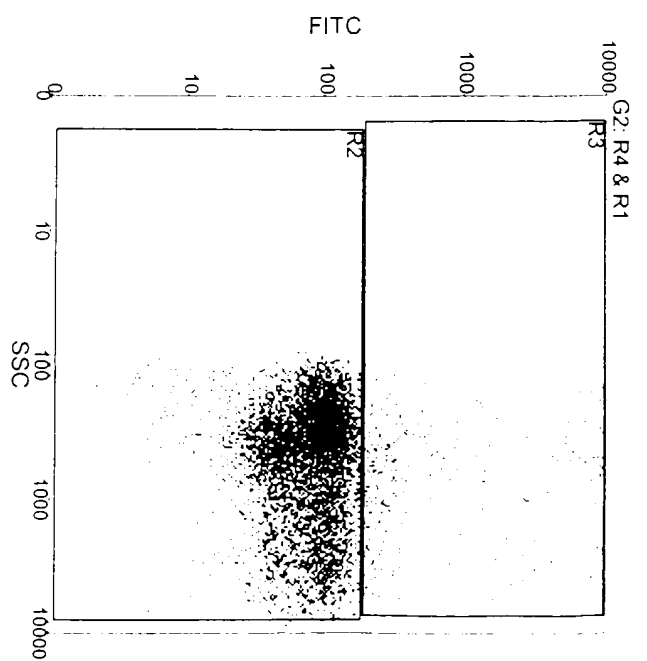
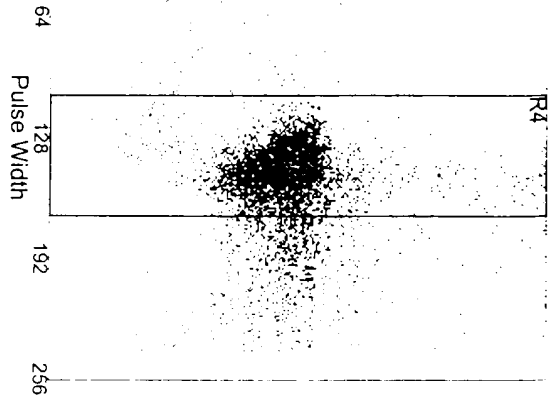
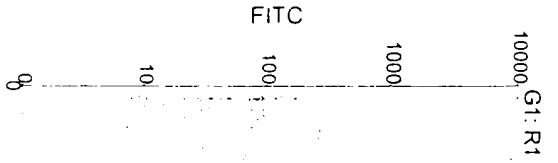
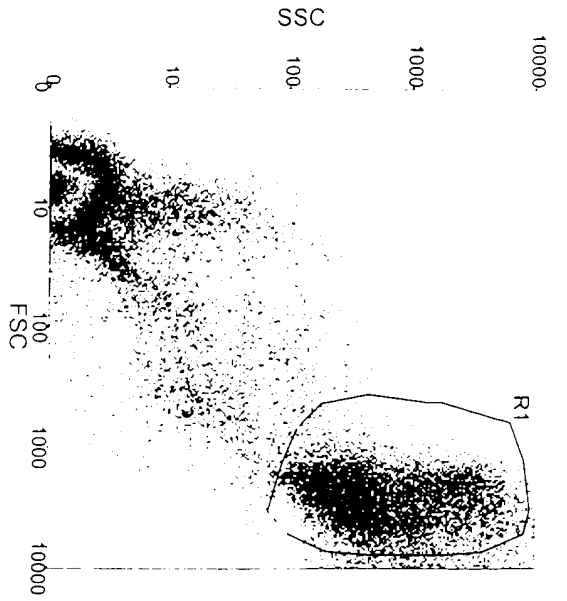
Region	Count	%	Mean
Total	10920	100.00	151.42, 247.95
R4	9111	83.43	140.98, 226.57



Region	Count	%	Mean
Total	9111	100.00	869.49, 226.57
R2	7448	81.75	876.97, 90.06
R3	1627	17.86	837.18, 648.93



Region	Count	%	Mean
Total	9111	100.00	226.57

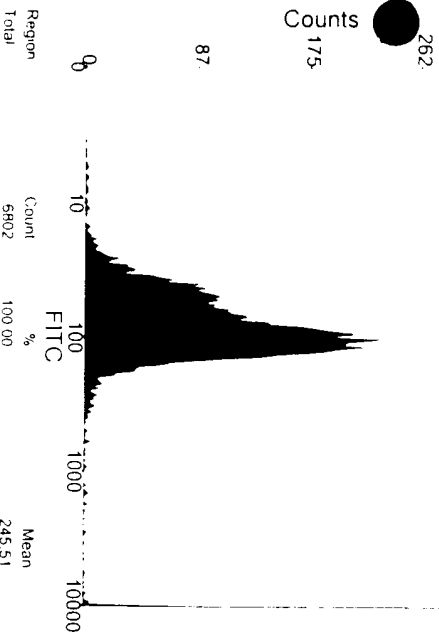


Region	Count	%	Mean
Total	50000	100.00	619.08, 197.16
R1	8650	17.32	2737.25, 902.77

Region	Count	%	Mean
Total	8695	100.00	157.27, 278.34
R4	6802	78.23	142.89, 245.51

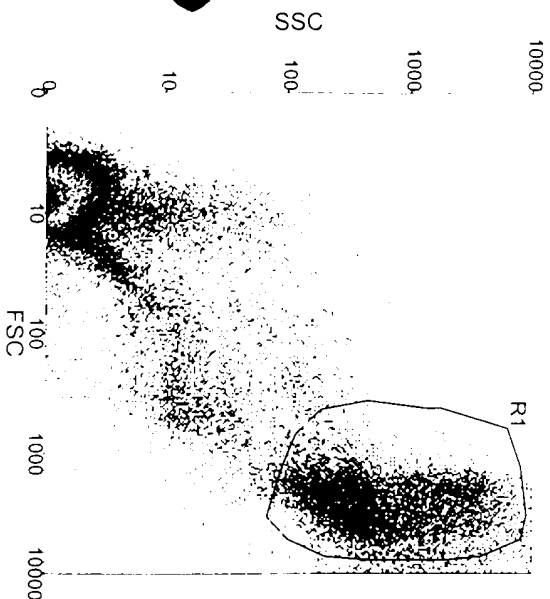
Region	Count	%	Mean
Total	6802	100.00	870.77, 245.51
R2	6438	94.65	850.34, 85.49
R3	286	4.20	1055.34, 1317.32

350 G2: R4 & R1

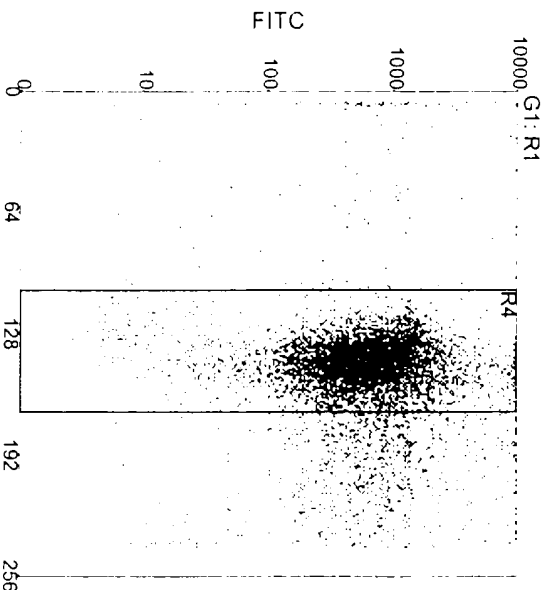


Counts  
262  
175  
87

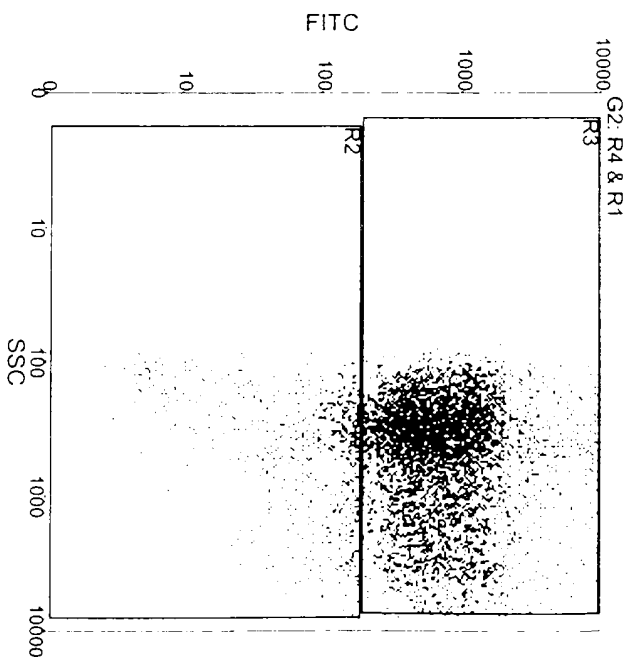




Region	Count	%	Mean
Total	50700	100.00	516.58, 195.81
R1	8956	17.93	2723.48, 879.04

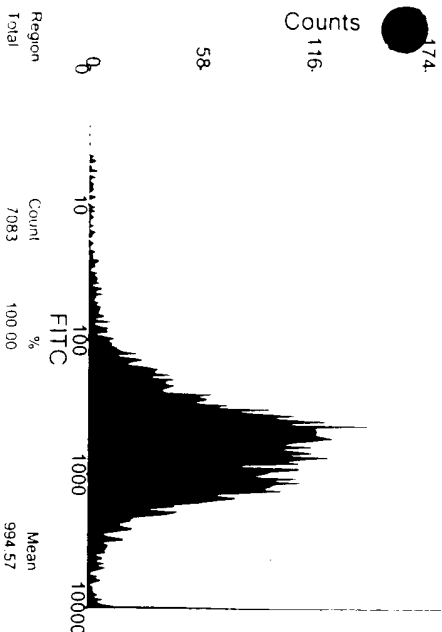


Region	Count	%	Mean
Total	8994	100.00	156.57, 1082.71
R4	7083	78.75	142.66, 994.57

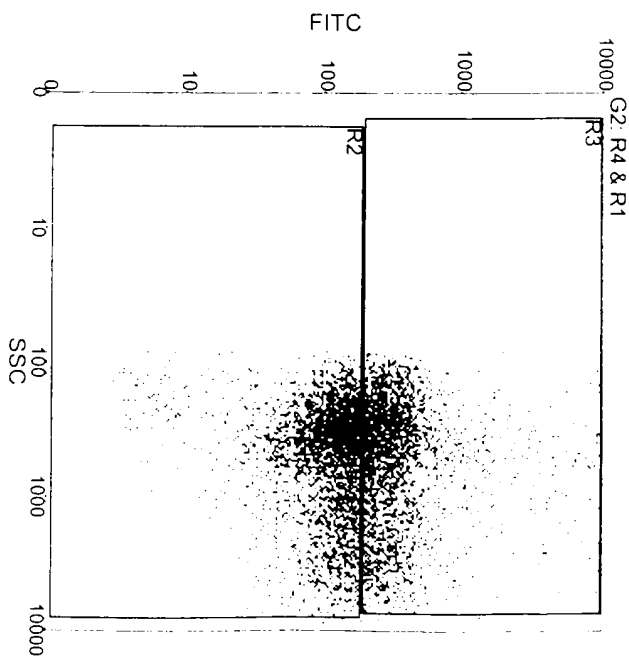
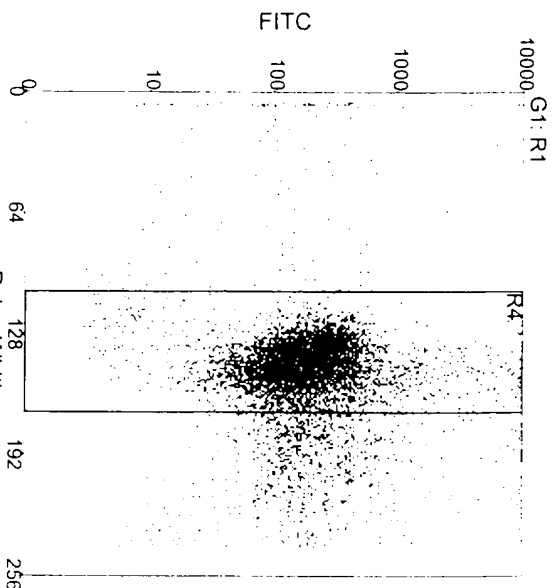
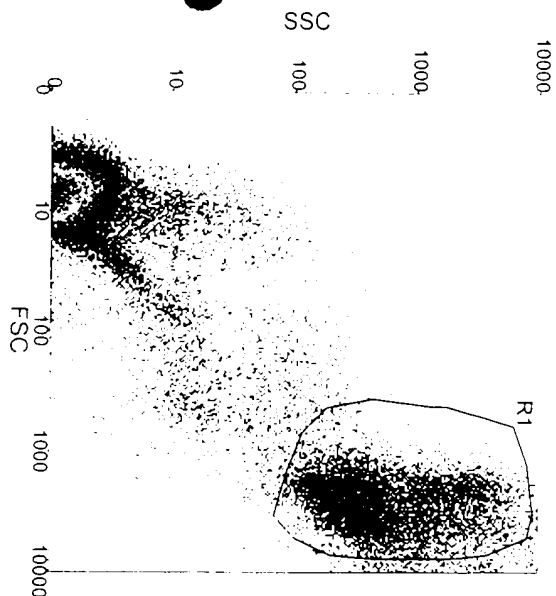


Region	Count	%	Mean
Total	7083	100.00	845.48, 994.57
R2	759	10.72	764.37, 104.30
R3	6211	87.69	852.70, 950.42

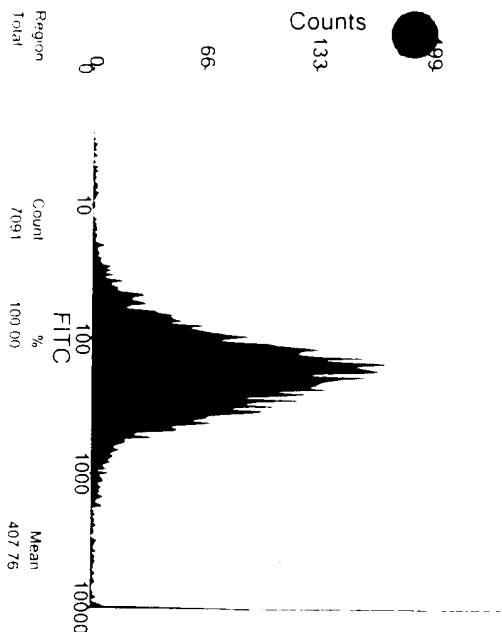
232: G2: R4 & R1

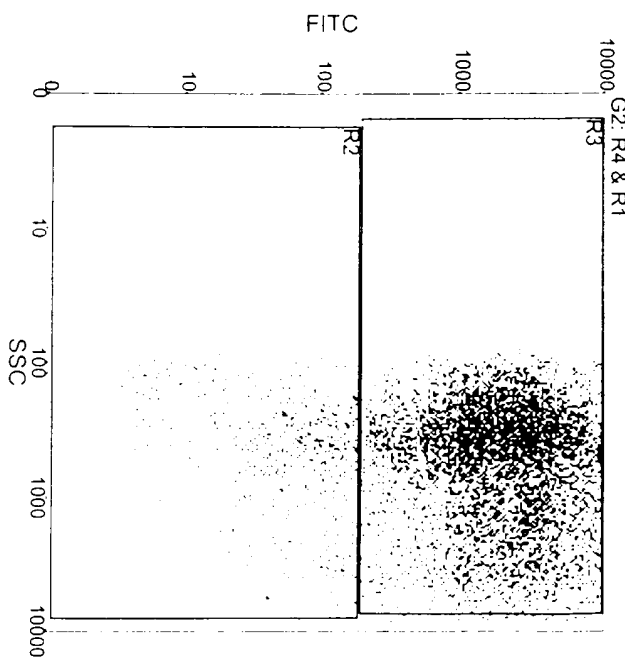
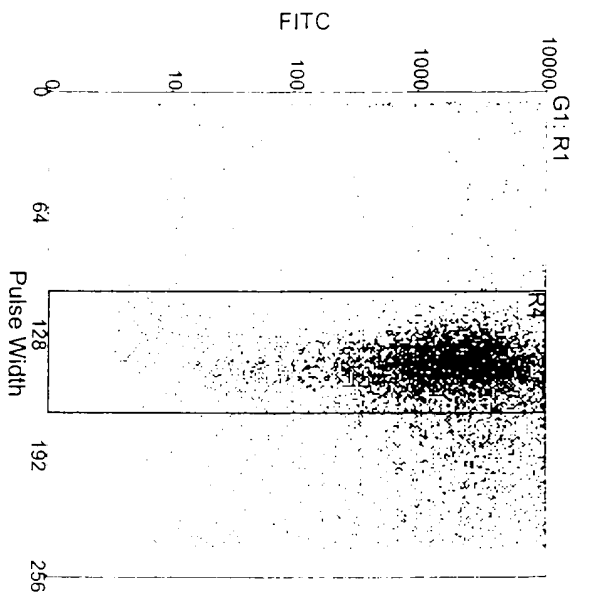
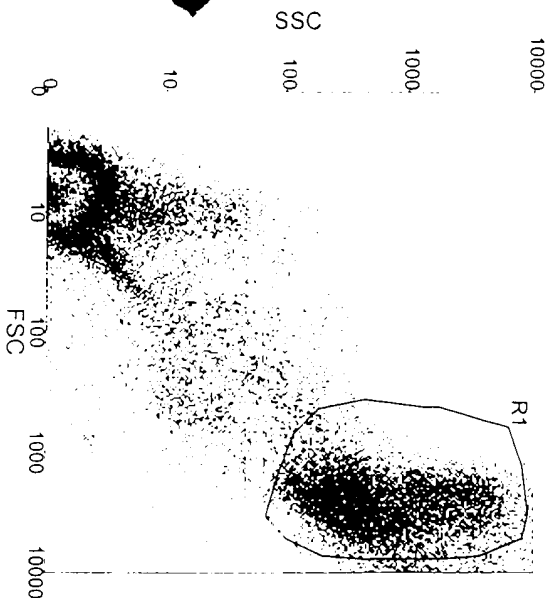


Region	Count	%	Mean
Total	7083	100.00	994.57

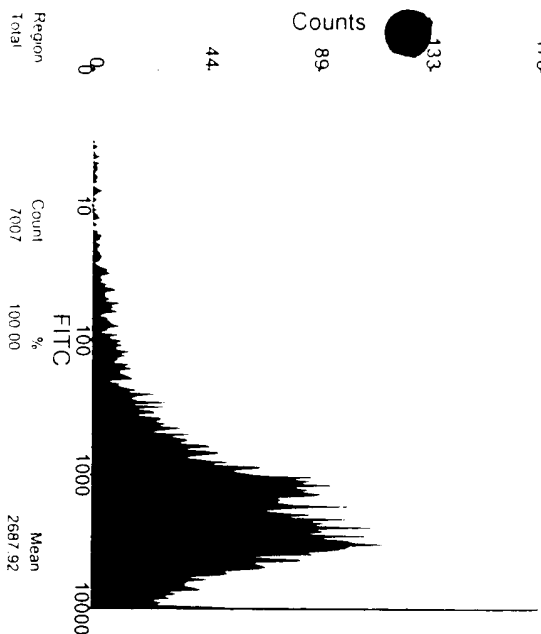


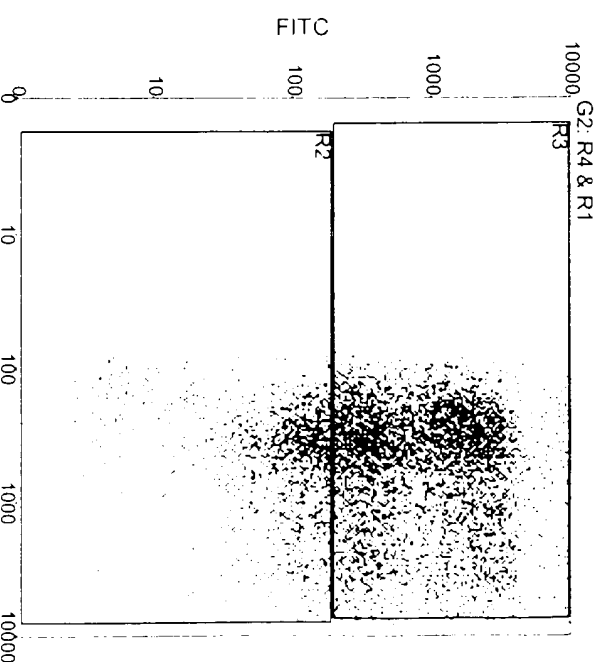
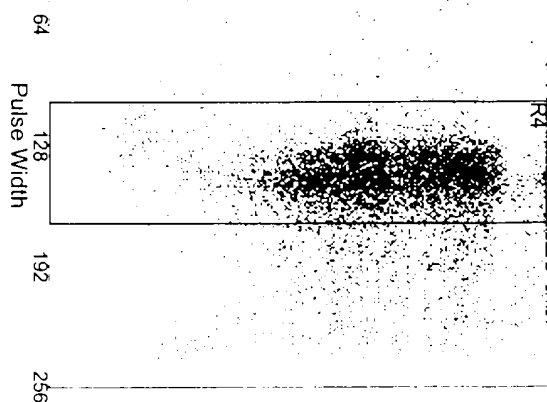
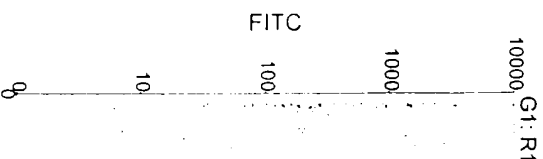
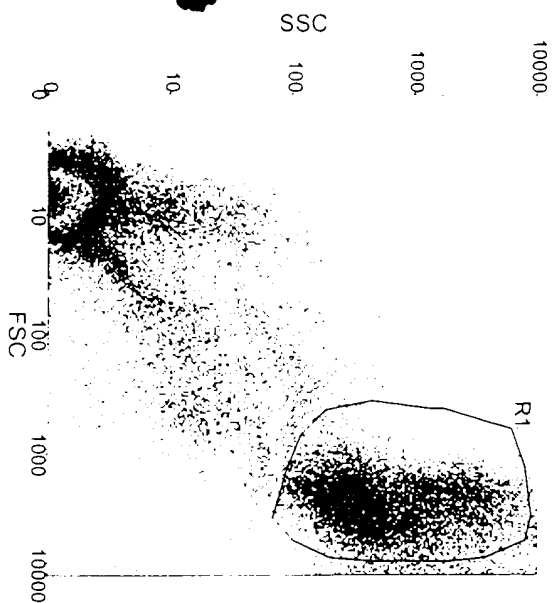
266 G2: R4 & R1





178 G2: R4 & R1



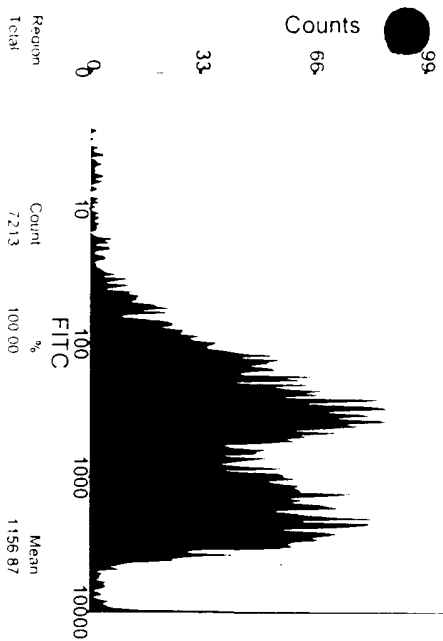


Region	Count	%	Mean
Total	50000	100.00	609.38, 197.20
R1	9020	18.04	2716.36, 880.21

Region	Count	%	Mean
Total	9050	100.00	155.80, 1212.35
R4	7213	79.70	142.79, 1156.87

Region	Count	%	Mean
Total	7213	100.00	867.66, 1156.87
R2	1636	22.68	790.21, 103.34
R3	5467	75.79	888.59, 1304.66

133 G2: R4 & R1

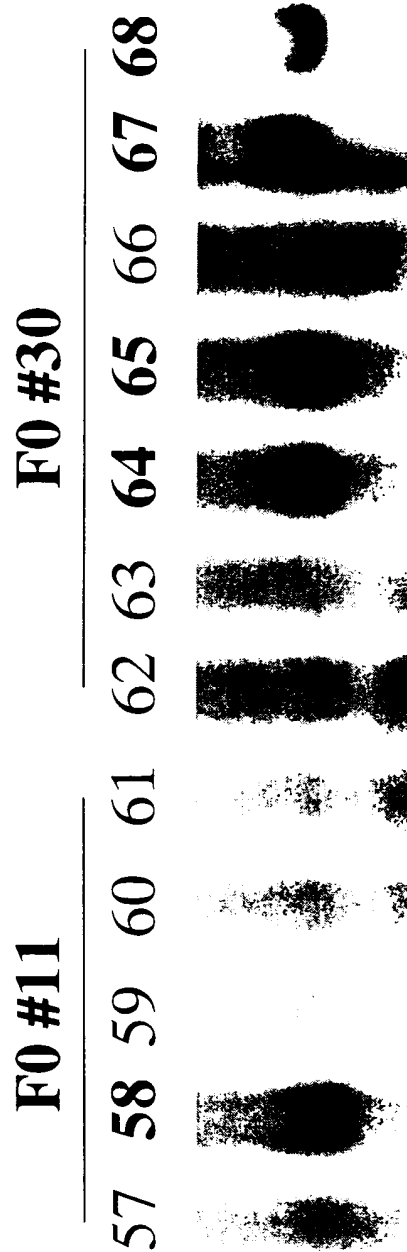


Region	Count	%	Mean
Total	7213	100.00	1156.87

Generation of Transgenic Mice from Two Different Linkers mAb  
C and mAb D

# Transgenic Mice Generated from mAb D Linker

## by Southern Blot Analyses



Date: November 17, 2000

# Transgenic Mice Generated from mAb C linker by Southern Blot Analyses

F0 #46  
└───┬───┐  
30 32 33



Date: February 8, 2001